No distinct stratification of ingesta particles and no distinct moisture gradient in the fore-stomach of non-ruminants: The wallaby, peccary, hippopotamus, and sloth

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No distinct stratification of ingesta particles and no distinct moisture gradient in the fore-stomach of non-ruminants: the wallaby, peccary, hippopotamus, and sloth

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Abstract

Herbivores that digest plant material in the fore-stomach can be divided in ruminants and non-ruminants. This study describes the distribution of feed particles (and inorganic material) and dry matter (DM) in the digestive tract of non-ruminant foregut fermenters. Results from passage trials led us to hypothesize that specific particle-sorting mechanisms, as observed in ruminants, are unlikely in non-ruminants. Therefore, no systematic particle size distribution effects (indicative of a sorting mechanism) should be evident in the fore-stomachs of these animals, but differences in fluid and particle retention suggest that differences in fluid concentration (measured as DM) could occur in the foregut of macropods and hippos. The gut content of eleven Bennett's wallabies (*Macropus rufogriseus*), six collared peccaries (*Pecari tajacu*), three pygmy hippos (*Hexaprotodon liberiensis*), two common hippos (*Hippopotamus amphibius*) and one two-toed sloth (*Choloepus didactylus*) were analysed with an emphasis on the fore-stomach. The ventral and dorsal regions in sacciform compartments, and peripheral and central regions in tubular compartments, were examined. Results were not uniform across the species studied. A potential sedimentation mechanism was observed firstly by the accumulation of sand in the fore-stomach of the peccary and sloth, and secondly by the lower DM content in peripheral versus central and ventral versus dorsal regions of the fore-stomach of the wallabies and common hippos, respectively. However, pair-comparisons for different gut regions of wallabies and peccaries yielded no differences in mean particle size between fore-stomach regions. To conclude, some digesta fractionation does occur in the fore-stomach of the studied groups of non-ruminants, but not in a uniform manner, which in turn is in accordance with morphological dissimilarities of their respective foregut structures. The absence of systematic fractionation effects in non-ruminant foregut fermenters emphasizes the innovative character of the sorting mechanism in ruminants.

Keywords

Foregut fermenters, particle size distribution, sorting, solute, macropods

Introduction

Herbivore digesta generally consists of a matrix or a suspension of different sized particles. In order to maximize the utilization of cellulose-based carbohydrates or microbial proteins, physiological mechanisms can operate to retain and concentrate certain sizes of particles, flush the digesta matrix with fluids, or retain fluids (and very fine particles) in particular segments of the gut. Such separation mechanisms operate for example in the reticulo-rumen – the fore-stomach of ruminants –, and in the large intestine of herbivorous rodents. They are usually linked to two empirical findings in digestive studies: First, there is a difference in the particle and solute retention time (Müller et al., 2011), and in the retention time of different-sized particles (Lechner-Doll et al., 1990; Schwarm et al., 2008; Clauss et al., 2011). Second, there is a distinct difference in the particle size distribution between different sections of the same gastrointestinal tract compartment, especially in the fore-stomach of camelids and ruminants (Lechner-Doll & von Engelhardt, 1989; Hummel et al., 2009) and in the large intestine of herbivorous rodents (Vispo & Hume, 1995). Additionally, there are differences in the moisture content between different fore-stomach segments of the same compartment, such as in ruminants (Hummel et al., 2009; Clauss et al., 2009a).

The anatomical structure of fore-stomachs can be of very different shape, diameter and volume. Thus, morphological and systematic groups of foregut-fermenters are each characterized by innovative characters. The 'key innovation' sensu Heard & Hauser (1995) of a sorting mechanism may have led to the high species diversity in extant ruminants as opposed to the generally lower species diversity of non-ruminant foregut fermenters (Langer, 1991; 1994). The sorting mechanism results in a dramatically improved digesta particle size

75 reduction, and hence may also facilitate generally higher intake levels (Schwarm et al., 2009a;
76 Clauss et al., 2010).

77 Non-ruminant foregut fermenters show variation in retention patterns of the various
78 digesta components – solutes, small and large particles. In macropods, peccaries and hippos,
79 solutes pass out of the fore-stomach faster than particles (Dellow, 1982; Clauss et al., 2004;
80 Schwarm et al., 2009b), in sloth solutes pass out of the fore-stomach slower than particles
81 (Foley et al., 1995), whereas no such difference is evident in colobine monkeys (Schwarm et
82 al., 2009b). Differences in the retention of smaller versus larger particles occurred
83 sporadically in peccaries, pygmy hippos and common hippos (Clauss et al., 2004; Schwarm et
84 al., 2008; Schwarm et al., 2009b), and were not evident at all in macropods (Schwarm et al.,
85 2009b; Munn et al., 2012) or colobine monkeys (Schwarm et al., 2009b). Given these
86 findings, we would expect corresponding variation in the composition of digesta from
87 different fore-stomach regions of these groups.

88 Particle size distribution in the fore-stomach digesta of non-ruminant foregut
89 fermenters has not been investigated systematically. The blindsac(s) or sacciform part(s) of
90 the fore-stomach of Macropodidae, Tayassuidae, Hippopotamidae and Bradypodidae has been
91 proposed as a kind of sedimentation trap(s) for smaller, denser particles (kangaroo: Lentle et
92 al., 2002; peccary: Schwarm et al., 2010; hippo: Langer, 1988, p429, Wings et al., 2008;
93 sloth: Clauss, 2004). Correspondingly, Langer (1976) and Foley et al. (1995) found higher
94 proportions of small particles in ventral compared to dorsal regions of the sacciform fore-
95 stomach compartments of free-ranging common hippos (N=2) and three-toed sloths (N=6),
96 respectively. Because sloth spend most of their time resting in a perched-sitting position, fore-
97 stomach sections are submitted to quite similar physical conditions as in other quadrupedal
98 mammals (reviewed by Clauss, 2004). No conclusions about separation mechanisms can be
99 reached from other studies because foregut compartments were only sampled as a whole but

not according to specific fore-stomach sub-sections (macropods: Freudenberger, 1992; Lentle et al., 2002 ; peccaries: Langer, 1979; common hippos and three-toed sloths: Langer, 1988).

In ruminant foregut fermenters, selective particle retention operates with a density-dependent flotation-sedimentation mechanism (Lechner-Doll et al., 1991), which requires a fluid environment. Therefore gradients in dry matter (DM) (or moisture) concentration can indicate sites of sedimentation-based sorting mechanism (within the reticulo-rumen: Clauss et al., 2009a; between fore-stomach compartments in non-ruminants: Langer, 1988; Foley et al., 1995). Another sorting mechanism might be based on the presence, and peristaltic movement, of haustra, as described for the rodent colon where solutes and fine particles are separated from coarser contents (Vispo & Hume, 1995). For the haustrated tubular fore-stomach of macropods, a propulsive peristalsis has been suggested, selectively transporting solutes and presumably fine particles towards the lower digestive tract (Langer, 1988, p 363; reviewed in Munn et al., 2012).

It was the aim of this study to compare mean particle size, size distributions of digesta particles, and DM concentration within fore-stomach compartments of Bennett's wallabies (*Macropus rufogriseus*), collared peccaries (*Pecari tajacu*), pygmy hippos (*Hexaprotodon liberiensis*), common hippos (*Hippopotamus amphibius*) and two-toed sloths (*Choloepus didactylus*). Gradients in moisture concentration within and between fore-stomach compartments in macropods and hippos would correspond to the clear distinction in the passage of solutes and particles in these species. In contrast, because of the absence of a consistent sorting mechanism for different-sized particles in any non-ruminant foregut fermenter, mean particle size of digesta sampled from ventral (or peripheral) regions of fore-stomach compartments were expected to be similar with those from dorsal (or central) regions, respectively.

125 **Materials and methods**

126 Gut content samples were collected from 11 Bennett wallabies, 6 collared peccaries (all culled
127 as part of surplus population control), and 3 pygmy hippos, 2 common hippos and 1 two-toed
128 sloth (all died for various reasons unrelated to our study). With the exception of the wallabies,
129 which were free-roaming within the zoo site, samples originated from animals kept in typical
130 zoo enclosures. Wallabies were grazing and browsing *ad libitum* on grass paddocks with
131 trees, bushes, and native plants of temperate chalk downland. Because wallabies were
132 accustomed to the presence of visitors, the feeding behaviour was not restricted to the
133 crepuscule as in free-ranging wallabies (Lentle et al., 1998). Peccaries were kept in a group
134 of approximately 30 animals and were fed twice daily a mixed diet consisting of fruits,
135 vegetables, cooked potatoes, grass or hay supplemented three times a week with chicks,
136 cooked eggs, grains and concentrates. Pygmy and common hippos were offered twice daily a
137 hay diet and a mixture of fruits and vegetables. The diet of one common hippo was
138 supplemented with rhino and grazer pellets (approx. 15% of DM intake each). The sloth diet
139 consisted of approximately 66% vegetables, 33% pickled rice and wheat, browser pellets and
140 cooked egg. Body mass was measured except for 1 pygmy hippo and both common hippos.
141 Details of the animals are summarized in **Table 1**.

142 Because recently consumed food may produce a uniform distribution of particles
143 (Lentle et al., 2007), we attempted to harvest guts some time after (not during) a feeding
144 period. The wallabies were shot in the morning and kept with ropes in an upright position
145 until and during necropsy. The peccaries were killed by darting and exsanguination in the
146 morning before feeding, and were kept in sternal recumbency until necropsy. Necropsy of
147 wallabies and peccaries was performed within 2 hours after death and ligatures were applied
148 to the various segments so as to prevent displacement of contents by gaseous products of in
149 situ fermentation. Hippos and sloth were submitted for necropsy by the respective zoological
150 gardens and removed guts were stored frozen at -20°C before harvesting of gut content.

Physiological positioning of guts during freezing (and thawing) was not guaranteed. The guts of hippos and sloth were filled but the time after feeding is unknown. Samples were taken from fresh or thawed gut content in the different fore-stomach compartments (each sampled ventral and dorsal in the case of sacciform structures or peripheral and central in the case of tubiform structures), from the glandular stomach, and the rectum. In peccaries, the upper blindsac of the fore-stomach was empty in most cases. Hippos and sloths lack a caecum, but in wallaby and peccary the caecum tip was sampled as well. In two out of three pygmy hippos the hindgut was not preserved for dissection, i.e. only one rectum sample was available. Samples were sealed watertight and stored frozen at -20°C until analysis.

Sub-samples were wet-sieved over a cascade of nine sieves of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm mesh size (Retsch VS 1000, Germany). Particles passing the finest sieve (representing also microorganisms) were discarded, and their proportion was estimated by the difference between the calculated DM weighed in for sieve analysis and the DM retrieved on the sieves. Five to 30 g of the thawed samples were suspended in tap water, poured over the sieve cascade and rinsed with one litre of water. Subsequent sieving time was 10 min at amplitude 45, with a water throughput of approximately 3 L/min. The particles of each sieve fraction were weighed (accuracy of 0.1 mg) after drying at 103°C to constant weight together with two subsamples for DM content determination. The size of the largest particle was measured manually. Because peccary and sloth samples contained macroscopically visible inorganic material, the whole sample and residuals from all sieves were, in total DM, corrected for acid insoluble ash (AIA) content. The AIA concentration was determined according to Van Keulen & Young (1977) by treatment of residual ash (550°C in a muffle furnace) with hydrochloric acid (25%). Additionally, we calculated the DM content in the non-AIA gut content (= moisture plus non-AIA DM), in order to correct results of DM content for the presence of sand.

For visualization of wet sieving results, the particle size distributions of digesta from various gut regions were plotted as frequency histograms. Two approaches were used to calculate the mean particle size of each sample from the retained dry weight on the respective sieves. The continuous mean particle size (cMEAN) was calculated from after fitting a suitable function to the respective cumulative sample data using TableCurve 2D v5.01 (Systat Software UK Ltd., UK) (x-axis: sieve mesh size, y-axis: cumulative percentage of retained particles) (Fritz et al., 2012). This method considers the area under the curve, which is limited by the maximum particle size. Sometimes, the chosen function did not include the manually measured maximum particle size, and a smaller size had to be accepted. The discrete mean particle size (dMEAN, mm) was calculated by multiplying the proportion of particles retained on each sieve (in % of the mass of particles retained on all sieves) with the calculated average mesh size, then adding up all these products, i.e. [0.06 x (0.063 mm + 0.125 mm / 2)] + [0.15 x (0.125 mm + 0.25 mm / 2) + etc. + [0.02 x (16 mm + manually measured maximum particle size / 2] (Fritz et al., 2012). Results for cMEAN and dMEAN were compared by linear regression analysis (95% confidence intervals are given). A good agreement of the two methods was evident (r^2=0.96, $p<0.001$, y=0.61 (0.57, 0.66) x + 0.64 (0.10, 1.17). In the following only the results of cMEAN are presented.

Results were analyzed by repeated measurements ANOVA, followed by subsequent pair-wise comparisons with Dunn–Sidak adjustment. For comparison of two compartments within species or between species, normally distributed data (Kolmogorov-Smirnov-test) were analysed by a Students paired t-test. Within species, monotonous associations between pairs of variables were assessed by calculating Spearman's rank correlation coefficients (SCC). All analyses were performed using IBM SPSS Statistics 19 (IBM, Germany). The significance level was set to α=0.05 and *P* values between 0.05 and 0.10 were considered as trends.

Results

The average DM concentration in different gut regions is presented in **Table 1**, and for peccary and sloth the AIA concentration of dried gut content and the estimated DM concentration of non-AIA gut content is given in the same table. The concentration of AIA in the peccary gut ranged from 20-94% DM and in the sloth gut from 5-13% DM. The mean particle size is depicted in **Table 2** and is summarized together with the DM content in gut drawings in **Figure 1**. Significant results of the statistical analyses are given in **Table 3**.

Comparing the average DM between the rectum and fore-stomach samples, DM in the former was approximately twice as high in wallaby (22 vs. 12%) and sloth (36 vs. 16%). In sloth this is consistent if DM of non-AIA content is compared. In peccaries, the presence of sand led to a high DM in both rectum and fore-stomach samples (64 vs. 58%), but a difference in DM was evident if non-AIA content was compared (32 vs. 13%). In contrast, in pygmy and common hippo no difference in DM between rectum and fore-stomach samples was evident.

In wallabies, DM was significantly lower in the ventral than dorsal region of the sacciform fore-stomach and tended ($p=0.082$) to be lower in peripheral than central regions of the tubiform fore-stomach. In common hippos, DM was lower in the ventral than dorsal region of the tubiform fore-stomach; interestingly DM was exactly the same in each regions of this compartment for both animals, although animals originated from different zoological institutions and were fed with or without a pelleted diet component. In contrast, DM was on average higher in the ventral than dorsal region of the blindsac(s) or did not differ between regions in this sacciform compartment in peccaries ($p=0.752$), pygmy hippos, common hippos, and sloth. In peccary the pattern is consistent if DM of non-AIA content is compared ($p=0.316$).

Particle size frequency histograms for the gut regions of each species are depicted in **Figure 2**. In wallaby, a relatively low variation in the relative proportions of each particle

fraction in the different gut compartments was observed compared to the other species. Post-pylorus particle size reduction was particularly evident in pygmy hippo and sloth. In the glandular stomach of the pygmy hippos, 26% (N=3) of the food particles was 0.063-0.500 mm small, whereas in the rectum 48% (N=1) of the food particles had this range of size. In the sloth these values were 8 and 48%. Accordingly, also the concentration of digesta material finer than 0.063 mm was particularly high in the rectum as compared to the glandular stomach of the pygmy hippo (52 vs. 13%) and sloth (77 vs. 6%; **Fig. 3**).

In peccaries, mean particle size in the anterior blindsac was smaller than in the connecting fore-stomach compartment. In the connecting compartment of the fore-stomach of wallaby, peccary, pygmy hippo and sloth (but not in common hippo), mean particle size was on average smaller than in the glandular stomach. Neither in wallabies, nor in peccaries was the mean particle size different between ventral and dorsal, or central and peripheral regions of the same fore-stomach compartment (**Table 3**). In pygmy hippos the mean particle size was on average smaller in the ventral than dorsal region of the visceral blindsac ($p=0.023$ using a Students paired t-test; but $p>0.05$, RM ANOVA and Sidak adjustment, all compartments).

Discussion

This study describes the distribution of feed particles (and sand) and solutes in the digestive tract of non-ruminant foregut fermenters. In accord with the morphological and physiological dissimilarity of their respective foregut structures, results were not uniform across these groups. A common evaluation of fore-stomach anatomy and functional measurements was exacerbated by the presence of sand in two of five species. Nevertheless, the presented data allow some conclusions on differences in the digestive physiology between these non-ruminant foregut fermenters.

253 *Inorganic material in the gut*

254 Similar to the finding of Wings et al. (2008) on inorganic material in the fore-stomach
255 blindsacs of captive pygmy hippopotamuses, the increased accumulation of sand in the
256 ventral fore-stomach compartments of both, peccaries and sloths, confirms the assumption
257 that fore-stomach structures may act as sedimentation traps for dense material (Clauss, 2004;
258 Schwarm et al., 2010). In contrast, sand was neither observed in the fore-stomach blindsacs of
259 the two captive common hippos of this study, nor in eleven common hippos from Kruger
260 National Park, South Africa (Langer, 1976, pers. comm.). The absence of soil accumulation in
261 the common hippo, a species foraging close to the ground, could indicate a specific adaptation
262 to deal with accidental soil ingestion. The relatively thick layer of smooth muscle (*Tunica*
263 *muscularis externa*) of the blindsac wall of common compared to pygmy hippos (A.
264 Schwarm, pers. observation) may allow forceful contractions that churn the sediment out of
265 this compartment.

266 Large amounts of sand in the gastrointestinal tract of peccaries have also been reported
267 by Langer (1979). In our study, we found even higher concentrations than this author (up to
268 94 vs. 70 %, **Fig. 4**). Schwarm et al. (2010) even suggested that the propensity for the
269 peccary's fore-stomach to trap soil might be a reason why this peculiar fore-stomach design
270 was not associated with a large species radiation, as for example the ruminant or macropod
271 fore-stomach design. The function of sand in terms of particle size reduction by grinding plant
272 material seems unlikely, given the low shear rates generated during alimentary pulsion and
273 retropulsion (Lentle et al., 2002) and the expected deleteriously impact of any such action
274 upon the mucosa. Nevertheless, in the peccary sample of this study, there was actually a
275 negative relationship between the AIA content in a fore-stomach compartment and the
276 particle size of its organic material ($n=27$, $SCC=-0.78$, $p<0.001$). No such relationship
277 between AIA content and mean particle size of organic material was evident in the sloth (with
278 the smaller sample size of $n=4$, $SCC=-0.20$, $p=0.800$).

279

280 *Faecal particle size*

281 Mean faecal particle size of the studied animals increased with increasing body mass, but did
282 not differ from non-ruminants given in the literature (**Fig. 5 a**). These findings support the
283 observation of Fritz et al. (2009) that foregut fermentation per se does not lead to smaller
284 digesta particles, but that dental adaptations and rumination are important in this respect.
285 Wallabies had on average 2.5-fold smaller particles in the faeces than peccaries of a
286 comparable body mass (0.76 vs. 1.94 mm, $p<0.001$). This difference is even greater when
287 particle size in the glandular stomach content is compared (0.97 vs. 6.74 mm, **Fig. 5 b**,
288 $p<0.001$). It is tempting to speculate that this difference in particle size is related to
289 differences in oral processing. The small digesta particles in wallabies would then potentially
290 be explained by the adaptation of teeth to shearing rather than crushing, by the extensive
291 initial feed comminution (Freudenberger, 1992) and by facultative mericysm, whereas
292 peccaries are constrained in their chewing movements due to their interlocking canines
293 (Langer, 1979). However, in this study, different diets may have contributed to differences in
294 particle size. Whether macropods are more efficient at ingestive particle size reduction than
295 other non-ruminant mammalian herbivores, should be investigated in a large sample of
296 species receiving a consistent diet.

297

298 *Differences along the intestinal tract*

299 In wallabies, peccaries and the sloth, DM concentration in the rectum was distinctively higher
300 than in preceding sections of the gastrointestinal tract, consistent with the general function of
301 fluid-reabsorption in the distal colon. In contrast, there was no marked increase in faecal DM
302 in either hippo species. Clauss et al. (2003) suggested that in hippos the capacious fore-
303 stomach does not allow sufficient space for a water-reabsorbing colon, hence limiting these
304 animals to an aquatic lifestyle.

305 The observed reduction of particle size from the stomach to the rectum in particular in
306 pygmy hippo and sloth could be due to diet components low in structural cell wall, and in
307 sloth also due to very long ingesta retention times (Foley et al., 1995) – similar to
308 observations on food low in structural fibre that is continuously reduced in particle size during
309 a long ingesta passage along the digestive tract of dugongs (Lanyon & Sanson, 2006).
310 Although the physical size degradation of particles beyond the fore-stomach is usually of low
311 magnitude in ruminants and macropods (Poppi et al., 1980; Freudenberger, 1992), we
312 observed finer digesta in rectum than fore-stomach contents in wallabies.
313 In wallaby, peccary, pygmy and common hippo, particle size in the connecting
314 compartment of the fore-stomach did not differ from the glandular stomach, but in the one
315 sloth studied, particle size was smaller in the former. For comparison, we calculated mean
316 particle sizes from sieving results of Freudenberger (1992) for Macropodinae (N=4: wallaby,
317 *Macropus robustus robustus*, and euro, *M. r. erubescens*) and of Foley et al. (1995) for
318 Bradypodidae (N=6, three-toed sloth, *Bradypus tridactylus*), assuming a maximal particle size
319 of 20 and 10 mm, respectively. In accord with our results, particle size in the connecting
320 compartment of the fore-stomach was the same as in the glandular stomach of the wallaby
321 (Freudenberger, 1992: 1.38 vs. 1.35 mm). In contrast to our result, particle size in sloth
322 studied by Foley et al. (1995) indicate no difference between these compartments (2.03 vs.
323 1.89 mm). The illustrated fore-stomach overview of Langer (1988, p 430) with the particle
324 classification, ‘fine’, ‘intermediate’ and ‘coarse’ does not increase consistency. Langer (1988)
325 depicted in the connecting compartment of Macropodinae, Tayassuidae and Hippopotamidae
326 larger (‘intermediate’/’coarse’) particles than in the glandular stomach (‘fine’). In
327 Bradypodidae particle size did not differ between these compartments (both ‘coarse’).
328 In wallabies, size distributions of digesta particles indicate an accumulation of smaller
329 particles in the caecum, but this was neither confirmed in the statistical comparison, nor in the
330 proportion of very fine particles. This is in accordance with the anatomy of the wallaby’s

large intestine, which is not haustrated. Therefore, selective transport of solute and fine particles, as in the colonic separation mechanism of rodents (Vispo & Hume, 1995), appears unlikely in this group. Similarly, in another wallaby species, particle size calculated from the data of Munn et al. (2009) indicate no accumulation of smaller particles in the caecum than rectum (1.01 vs. 0.77 mm) but the proportion of the eluate, i.e. particles passing the finest sieve (0.045 mm) was on average higher in the caecum than rectum (~29 vs. ~18%). In the peccary, in contrast, the fraction of very fine particles in the caecum was 2-fold higher as compared to the rectum (42 vs. 24%) and also an increase in moisture was apparent (9 vs. 32%). The peccary colon is haustrated (Schwarm et al., 2010), which opens the possibility for a retrograde transport of the solute and very fine particle fraction, even though investigations on the peristalsis of the colonic haustra in this species and even in domestic pigs are lacking.

Differences within the fore-stomach

The DM concentration (10-20%) of the fore-stomach digesta of wallabies, pygmy and common hippos, sloths and the corrected DM concentration of peccaries was within the range reported for the reticulo-rumen content of ruminants (up to 16% DM, Hummel et al., 2009; Clauss et al., 2009a). However, low DM concentrations of 5-8% in the reticulum of 'cattle-type' ruminants reported by the same authors, were not detected in the present study. This may indicate an absence of a flotation-sedimentation mechanism as well as differences in saliva production, which remains to be investigated in non-ruminant foregut fermenters.

Ingesta passage studies in non-ruminant foregut fermenters revealed a 3- to 4-fold faster passage of solutes than particles in macropods (Dellow, 1982; Schwarm et al., 2009b; Munn et al., 2012) and hippos (Clauss et al., 2004; Schwarm et al., 2008), a 1.6-fold faster passage of solutes than particles in peccaries (Schwarm et al., 2009b), but no such distinct separation in sloths (Foley et al., 1995). Therefore, we expected gradients in moisture concentration within and between fore-stomach compartments at least in macropods and

357 hippos. In ruminants, differences in the DM concentration between larger sections of the fore-
358 stomach could be demonstrated in live (cannulated) animals (Hummel et al., 2009; Lechner et
359 al., 2010) and after dissection (Clauss et al., 2009a; Clauss et al., 2009b). The difference in
360 DM concentration between the ventral and dorsal region of the blindsac in wallabies (10 vs.
361 13%) is comparable to the difference between ventral and dorsal rumen content of oxen (5-
362 9% vs. 8-14%, Hummel et al., 2009). Similarly, differences between the dorsal and ventral
363 content of the connecting chamber in common hippos indicate saturation with moisture
364 compatible with the observed difference in solute and particle marker excretion. In accord
365 with our expectation, fore-stomach compartments of peccaries and the sloth did not indicate a
366 moisture gradient. However, the absence of such a moisture gradient in the pygmy hippos, for
367 which a distinct separation of solute and particle ingesta has been demonstrated in passage
368 studies, suggests that the measurement of ingesta DM content after death may not yield
369 conclusive results in all cases - especially when guts could not be kept in physiological
370 position.

371 In macropods, radiological studies demonstrated active extrusion of the solute phase
372 along the gastric sulcus but poor reabsorption of solute into the digesta plug (Lentle et al.,
373 2002). Therefore, we had expected DM content to be lower in digesta sampled from the
374 periphery of the tubiform fore-stomach than from its centre. This difference existed
375 numerically but not statistically, suggesting that the function of haustra needs to be
376 investigated by other mechanisms than dissections of dead animals.

377 As predicted, mean particle size was not smaller in ventral and peripheral than dorsal
378 and central regions of the fore-stomach of wallabies, peccaries, pygmy and common hippos
379 and sloth. Only in the visceral blindsac of pygmy hippos, particle size was numerically (but
380 not significantly) smaller in the ventral than dorsal region. Langer (1976) found higher
381 proportions of small particles in the ventral than dorsal region of the visceral blindsac of the
382 one common hippo studied. In sloth, mean particle size calculated from sieving results of

383 Foley et al. (1995) revealed to be smaller in the ventral (central pouch, 1.7 mm) than dorsal
384 region (fundus/connecting compartment 1, 2.6 mm).

385 To conclude, some digesta fractionation does occur in the fore-stomach of the studied
386 groups of non-ruminants, but not in a uniform manner, which in turn is in accordance with
387 morphological dissimilarities of their respective foregut structures. The absence of systematic
388 fractionation effects in non-ruminant foregut fermenters emphasizes the innovative character
389 of the sorting mechanism in ruminants.

390

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396

397

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514 **Figure legends**

515 **Figure 1** Mean sizes of digesta particles (mm) and dry matter concentrations (%) in different
516 gut regions of Bennett's wallabies (*Macropus rufogriseus*), collared peccaries (*Pecari tajacu*),
517 pygmy hippos (*Hexaprotodon liberiensis),* common hippos (*Hippopotamus amphibius*) and
518 two-toed sloth (*Choloepus didactylus*). Drawing by Jeanne Peter.

519

520 **Figure 2** Size distributions (% retained dry weight) of digesta particles in the fore-stomach,
521 glandular stomach (Gla), caecum (Cae) and rectum (Rec) of Bennett's wallabies (*Macropus*
522 *rufogriseus*), collared peccaries (*Pecari tajacu*), pygmy hippos (*Hexaprotodon liberiensis*),
523 common hippos (*Hippopotamus amphibius*) and two-toed sloth (*Choloepus didactylus*).
524 Sacciform fore-stomach compartments (Sac1, Sac2) are differentiated in a dorsal and ventral
525 section. Tubiform or connecting fore-stomach compartments (Con) are differentiated in a
526 central and peripheral section (wallaby) or in a dorsal and ventral section (peccary, hippos).
527 Nomenclature follows Langer (1988, p 262): *Sacciform fore-stomach 1*: in peccary "anterior
528 blindsac", in hippo "visceral blindsac"; in sloth dorsal: "fundus/cranial part of central pouch",
529 ventral: "central pouch". *Sacciform fore-stomach 2*: in peccary "upper blindsac", in hippo
530 "parietal blindsac"; in sloth "fundus/ diverticulum". *Connecting fore-stomach* (either
531 sacciform or tubiform): in wallaby "tubiform fore-stomach", in peccary "gastric pouch", in
532 hippo "connecting chamber", in sloth "connecting pouch".

533

534

Figure 3 Concentration of digesta material finer than 0.063 mm (contains also microorganisms) in the fore-stomach, glandular stomach (Gla), caecum (Cae) and rectum (Rec) of Bennett's wallabies (*Macropus rufogriseus*, N=11), collared peccaries (*Pecari tajacu*, N=6), pygmy hippos (*Hexaprotodon liberiensis*, N=3), common hippos (*Hippopotamus amphibius*, N=2) and two-toed sloth (*Choloepus didactylus*, N=1). Mean (+ SD when N≥3). Sacciform fore-stomach compartments (Sac1, Sac2) are differentiated in a dorsal (d) and ventral (v) section. Tubiform or connecting fore-stomach compartments (Con) are differentiated in a central (c) and peripheral (p) section (wallaby) or in a dorsal and ventral section (peccary, hippos).

Figure 4 Concentration of inorganic material in the gastrointestinal tract of captive collared peccaries (*Pecari tajacu*, this study N=6, mean±SD; Langer 1979 N=2) and one two-toed sloth (*Choloepus didactylus*, this study N=1). In the study of Langer (1979) "sand was carefully collected out of the different samples and weighed". In our study, acid insoluble ash content was determined in dried total gut content.

Figure 5 a) Relationship between body mass and mean faecal particle size in non-ruminant foregut fermenters (Data from Table 1, *Macropus rufogriseus*, *Pecari tajacu*, *Hexaprotodon liberiensis*, *Hippopotamus amphibius*, *Choloepus didactylus*) and hindgut fermenters (mammalian herbivores; Data from Fritz et al., 2009) **b)** Relationship between body mass and mean particle size of glandular stomach content (Individual data from our study, *Macropus rufogriseus*, *Pecari tajacu*, *Hexaprotodon liberiensis*, *Hippopotamus amphibius*, *Choloepus didactylus)*.

561 **Table legends**

562 **Table 1** Animal details, dry matter concentration (DM, %) of gut content (as sampled), acid
563 insoluble ash (AIA, %) concentration of dried gut content and DM (%) of non-AIA gut
564 content from different gut regions of Bennett's wallaby (*Macropus rufogriseus*), collared
565 peccary (*Pecari tajacu*), pygmy hippo (*Hexaprotodon liberiensis),* common hippo
566 (*Hippopotamus amphibius*) and sloth (*Choloepus didactylus*). Mean ± SD or Mean (individual
567 values when N=2).

568

569 **Table 2** Mean particle size (cMEAN, mm) of digesta from different gut regions of Bennett's
570 wallaby (*Macropus rufogriseus*), collared peccary (*Pecari tajacu*), pygmy hippo
571 (*Hexaprotodon liberiensis),* common hippo (*Hippopotamus amphibius*) and sloth (*Choloepus*
572 *didactylus*). Mean ± SD or Mean (individual values when N=2).

573

574 **Table 3** Results of the Repeated Measurements ANOVA models: *P* values (<0.05) for pair-
575 wise comparisons with Dunn–Sidak adjustment of mean particle size and dry matter (DM)
576 concentration of digesta in gut compartments of Bennett's wallaby (*Macropus rufogriseus*,
577 N=11) and collared peccary (*Pecari tajacu*, N=6). For peccary samples also acid insoluble ash
578 (AIA) of dried gut content and DM of non-AIA gut content are given.

579

580

581

582 **Table 1**

Species		**Wallaby**	**Peccary**	**Pygmy hippo**	**Common hippo**	**Sloth**
N		11	6	3	2	1
Origin/Zoo		a	b	c, d	a, e	c
Body mass	(kg, Mean ± SD)	16.8 ± 4.8	21.1 ± 1.1	228, 218, 223[1]	1000[1], 2300[1]	1.9
Body mass	(kg, Min. - Max.)	6.7 - 24.4	19.0 - 22.0			
Dry matter (DM, %) of gut content (as sampled)						
Fore-stomach						
Sacciform 1	*dorsal*	12.9 ± 1.6	49.0 ± 21.8	17.8 ± 4.7	12.1 (14.7/9.4)	14.1
Sacciform 1	*ventral*	10.3 ± 1.3	63.0 ± 14.3	19.5 ± 2.2	9.6 (n.s./9.6)	16.5
Sacciform 2	*dorsal*	n.e.	38.2 (52.5/23.9)	15.6 ± 4.1	13.3 (17.3/9.2)	16.3
Sacciform 2	*ventral*	n.e.	83.2 (N=1)	16.2 ± 5.3	14.4 (18.9/9.9)	
Connecting	*dorsal / central*	12.9 ± 1.7	17.4 ± 3.3	17.2 ± 1.1	17.7 (17.7/17.6)	17.4
Connecting	*ventral/ peripheral*	11.2 ± 1.7	16.6 ± 3.2	16.6 ± 2.4	12.7 (12.7/12.7)	
Glandular stomach		11.8 ± 1.5	27.8 ± 12.0	15.4 ± 1.7	13.4 (13.2/13.6)	17.0
Caecum		10.7 ± 1.5	68.9 ± 14.7	n.e.	n.e.	n.e.
Rectum		22.1 ± 2.5	64.3 ± 10.4	19.4 (N=1)	12.7 (14.8/10.6)	35.7
Acid insoluble ash (AIA, %) of dried gut content						
Fore-stomach						
Sacciform 1	*dorsal*		86.6 ± 13.4			5.6
Sacciform 1	*ventral*		89.6 ± 5.1			9.7
Sacciform 2	*dorsal*		73.9 (81.4/66.3)			4.7
Sacciform 2	*ventral*		97.1 (N=1)			
Connecting	*dorsal*		18.3 ± 8.9			13.0
Connecting	*ventral*		22.3 ± 6.6			
Glandular stomach			61.0 ± 20.7			10.6
Caecum			93.5 ± 8.0			
Rectum			73.1 ± 9.9			5.8
DM (%) of non-AIA gut content (= moisture + non-AIA DM)						
Fore-stomach						
Sacciform 1	*dorsal*		9.4 ± 4.1			13.4
Sacciform 1	*ventral*		14.6 ± 3.8			15.1
Sacciform 2	*dorsal*		13.3 (17.1/9.6)			15.7
Sacciform 2	*ventral*		12.6 (N=1)			
Connecting	*dorsal*		14.5 ± 1.6			15.5
Connecting	*ventral*		13.4 ± 2.3			
Glandular stomach			11.0 ± 0.3			15.5
Caecum			9.3 ± 2.6			n.e.
Rectum			31.8 ± 3.4			34.3

[1]estimated; n.e.: not existent; n.s.: not sampled

Forestomach nomenclature follows Langer (1988, p 262):

Sacciform 1: in peccary "anterior blindsac", in hippo "visceral blindsac"; in sloth dorsal: "fundus/cranial part of central pouch", ventral: "central pouch".

Sacciform 2: in peccary "upper blindsac", in hippo "parietal blindsac"; in sloth "fundus/ diverticulum".

Connecting: in wallaby "tubiform fore-stomach", in peccary "gastric pouch", in hippo "connecting chamber", in sloth "connecting pouch"

583

584 **Table 2**

Species		Wallaby	Peccary	Pygmy hippo	Common hippo	Sloth
N		11	6	3	2	1
Mean particle size (mm)						
Fore-stomach						
Sacciform 1	*dorsal*	0.88 ± 0.23	2.13 ± 1.32	9.27 ± 1.94	11.90 (12.52/11.28)	2.71
Sacciform 1	*ventral*	0.97 ± 0.22	2.53 ± 1.48	6.87 ± 2.17	13.36 (n.s./13.36)	2.95
Sacciform 2	*dorsal*	n.e.	4.97 (2.74/7.21)	10.81 ± 5.07	18.24 (17.82/18.66)	2.00
Sacciform 2	*ventral*	n.e.	1.51 (N=1)	13.61 ± 6.43	22.30 (31.11/13.50)	
Connecting	*dorsal / central*	0.82 ± 0.27	6.19 ± 1.53	8.55 ± 2.47	11.65 (15.05/8.25)	1.86
Connecting	*ventral/ peripheral*	0.90 ± 0.24	5.32 ± 1.48	10.34 ± 4.41	11.97 (14.59/9.36)	
Glandular stomach		0.97 ± 0.43	6.74 ± 3.11	11.63 ± 2.80	11.45 (14.17/8.73)	2.99
Caecum		0.60 ± 0.14	2.73 ± 1.20	n.e.	n.e.	n.e.
Rectum		0.76 ± 0.10	1.94 ± 0.83	4.86 (N=1)	11.91 (13.16/10.65)	0.50

n.e.: not existent, n.s.: not sampled
Forestomach nomenclature follows Langer (1988, p 262):
Sacciform 1: in peccary "anterior blindsac", in hippo "visceral blindsac"; in sloth dorsal: “fundus/cranial part of central pouch”, ventral: "central pouch".
Sacciform 2: in peccary "upper blindsac", in hippo "parietal blindsac"; in sloth "fundus/ diverticulum".
Connecting: in wallaby “tubiform fore-stomach”, in peccary "gastric pouch", in hippo "connecting chamber", in sloth "connecting pouch"

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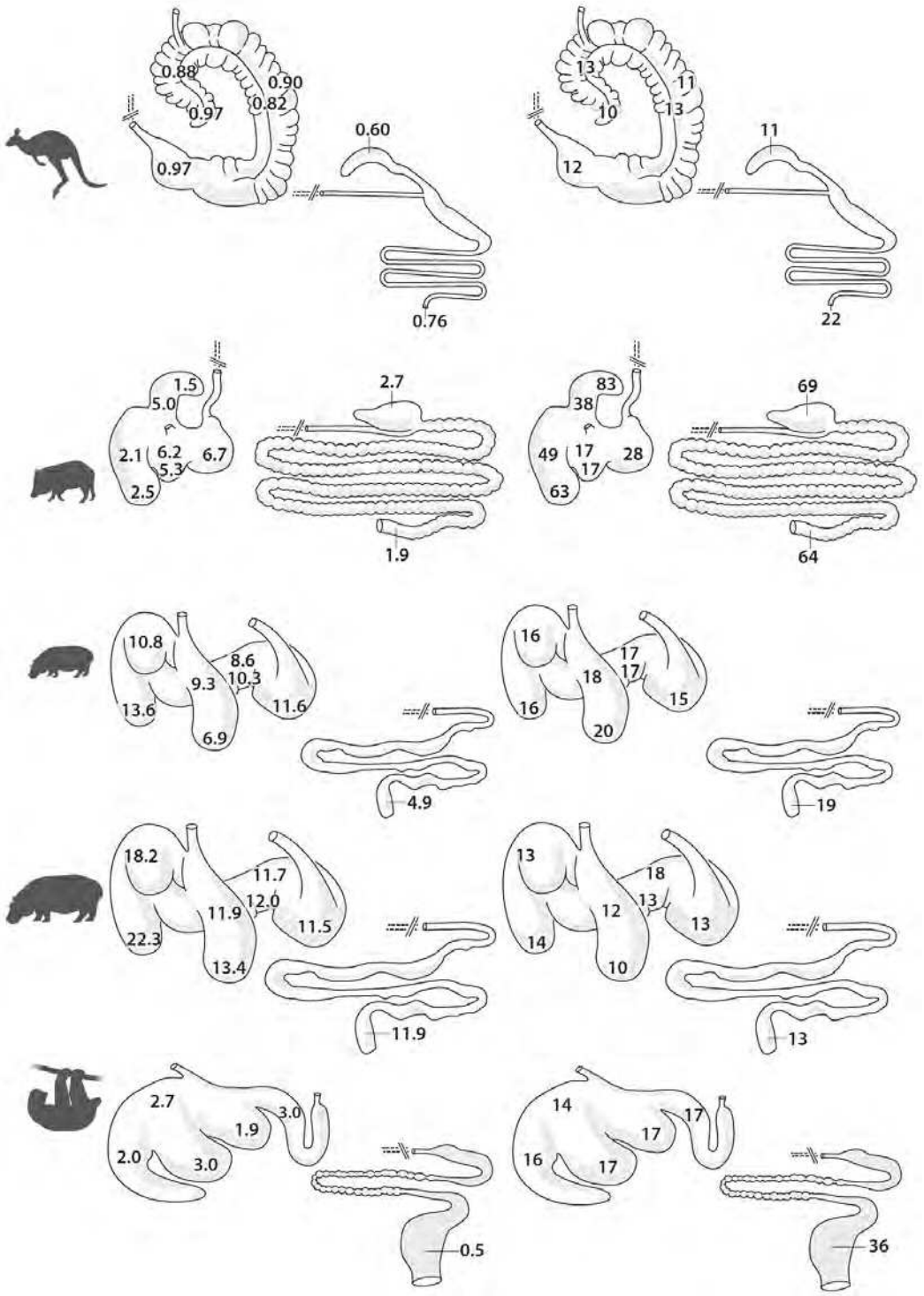
589 **Table 3**

Species	Gut compartment	Direction of effect	Gut compartment	P value
Dry matter (DM, %) of gut content (as sampled)				
Wallaby	Sacciform 1 dorsal	<	Rectum	<0.001
	Sacciform 1 ventral	<	Sacciform 1 dorsal	0.008
	Sacciform 1 ventral	<	Connecting central	0.003
	Sacciform 1 ventral	<	Rectum	<0.001
	Connecting central	<	Rectum	<0.001
	Connecting peripheral	<	Rectum	<0.001
	glandular stomach	<	Rectum	<0.001
Peccary	Sacciform 1 ventral	>	Connecting dorsal	0.016
	Sacciform 1 ventral	>	Connecting ventral	0.013
	Sacciform 1 ventral	>	Glandular stomach	0.036
	Connecting dorsal	<	Caecum	0.004
	Connecting dorsal	<	Rectum	0.001
	Connecting ventral	<	Caecum	0.005
	Connecting ventral	<	Rectum	0.001
	Glandular stomach	<	Caecum	0.045
Acid insoluble ash (AIA, %) of dried gut content				
Peccary	Sacciform 1 dorsal	>	Connecting dorsal	0.001
	Sacciform 1 dorsal	>	Connecting ventral	<0.001
	Sacciform 1 ventral	>	Connecting dorsal	0.001
	Sacciform 1 ventral	>	Connecting ventral	<0.001
	Connecting dorsal	<	Caecum	<0.001
	Connecting dorsal	<	Rectum	0.001
	Connecting ventral	<	Caecum	<0.001
	Connecting ventral	<	Rectum	0.004
DM (%) of non-AIA gut content (= moisture + non-AIA DM)				
Peccary	Sacciform 1 dorsal	<	Rectum	0.001
	Sacciform 1 ventral	<	Rectum	0.010
	Connecting dorsal	<	Rectum	0.001
	Connecting ventral	<	Rectum	<0.001
	Glandular stomach	<	Rectum	0.001
	Caecum	<	Rectum	<0.001
Mean particle size (mm)				
Wallaby	Sacciform 1 dorsal	>	Caecum	0.041
	Sacciform 1 ventral	>	Caecum	0.020
	Connecting peripheral	>	Caecum	0.047
Peccary	Sacciform 1 dorsal	<	Connecting dorsal	0.028
	Sacciform 1 ventral	<	Connecting dorsal	0.032
	Connecting dorsal	>	Caecum	0.019
	Connecting dorsal	>	Rectum	0.005
	Connecting ventral	>	Rectum	0.008

Forestomach nomenclature follows Langer (1988, p 262):
Sacciform 1: in peccary "anterior blindsac", in hippo "visceral blindsac"; in sloth dorsal: “fundus/cranial part of central pouch”, ventral: "central pouch".
Sacciform 2: in peccary "upper blindsac", in hippo "parietal blindsac"; in sloth "fundus/ diverticulum".
Connecting: in wallaby “tubiform fore-stomach”, in peccary "gastric pouch", in hippo "connecting chamber", in sloth "connecting pouch"

590

Figure 1


Mean particle size (mm)
Dry matter content (%)
0.88
0.90
0.97
0.82
0.97
0.60
0.76
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11
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12
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5.0
2.1
6.2
5.3
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2.5
2.7
1.9
83
38
49
17
17
28
63
69
64
10.8
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11.6
6.9
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2.0
3.0
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36

Figure 2

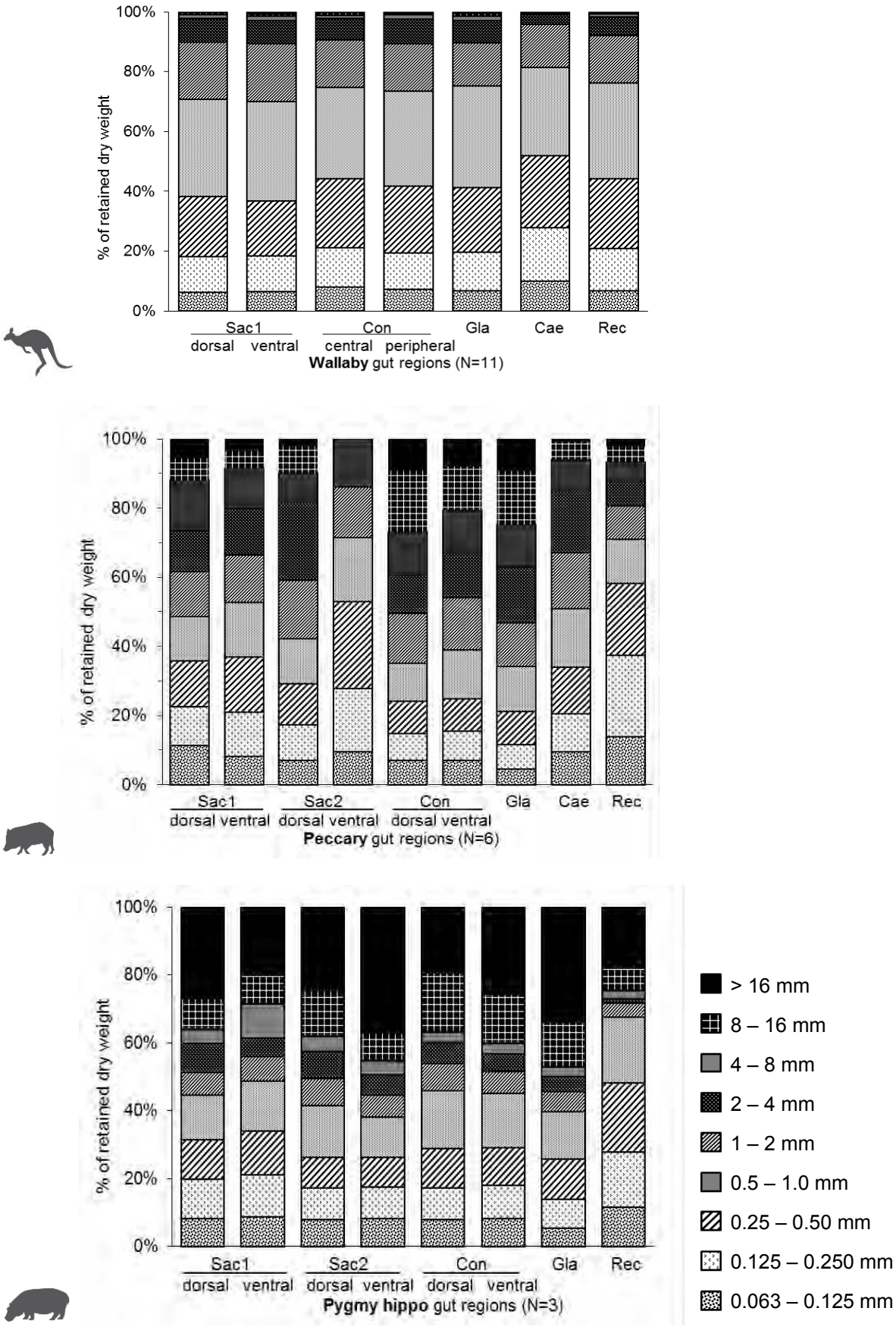
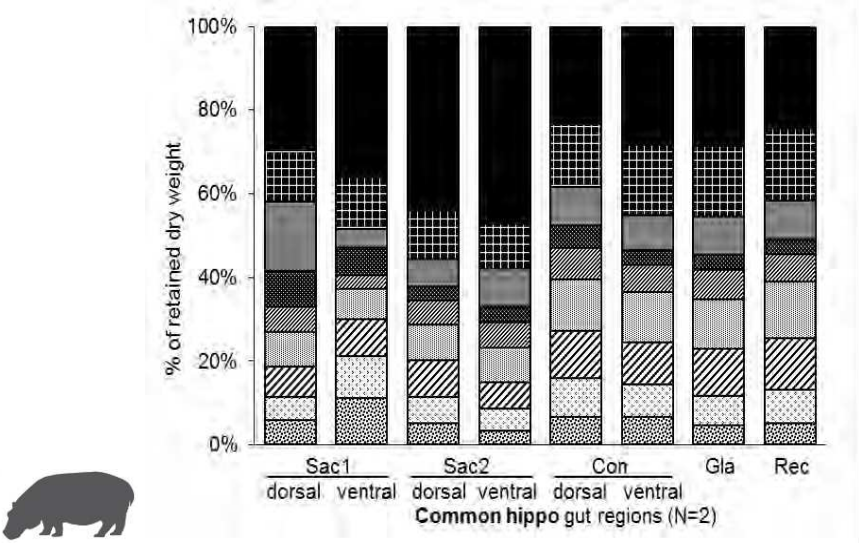

100%
80%
60%
40%
20%
0%
% of retained dry weight
Sac1
dorsal ventral
Con
central peripheral
Gla
Cae
Rec
Wallaby gut regions (N=11)
Sac2
Peccary gut regions (N=6)
Pygmy hippo gut regions (N=3)
> 16 mm
8 – 16 mm
4 – 8 mm
2 – 4 mm
1 – 2 mm
0.5 – 1.0 mm
0.25 – 0.50 mm
0.125 – 0.250 mm
0.063 – 0.125 mm

Figure 2 continued



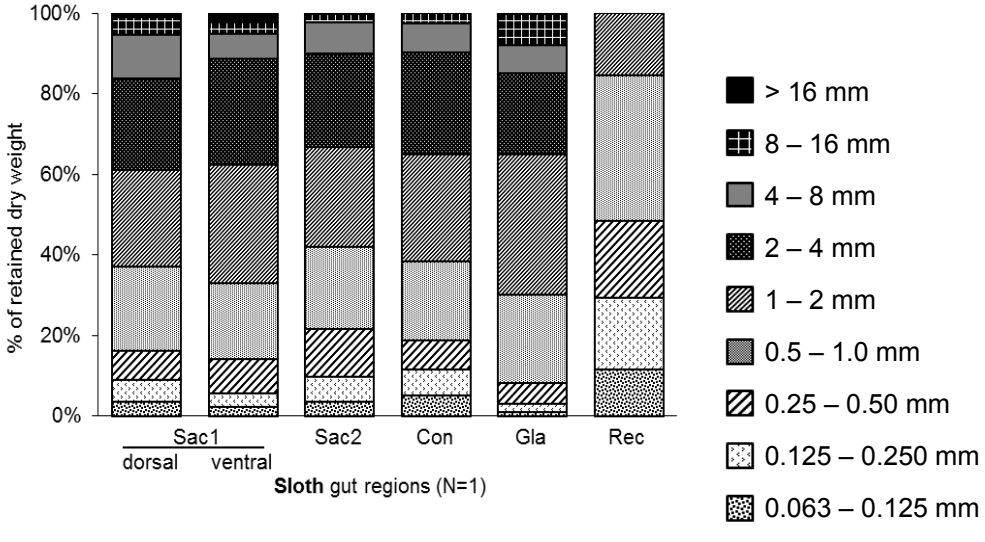


Figure 3

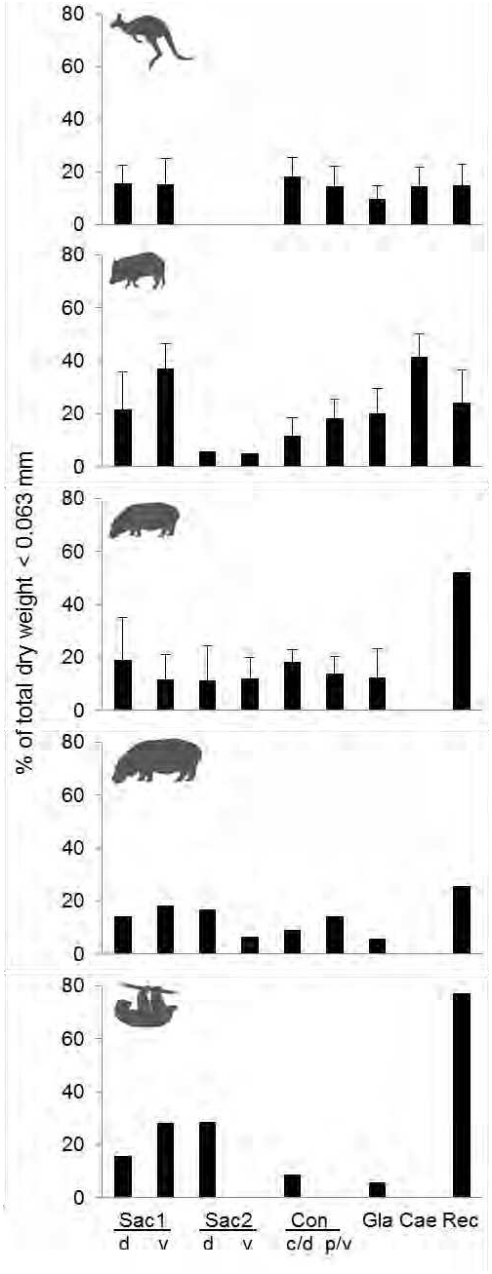

% of total dry weight < 0.063 mm
80
60
40
20
0
Sac1
d v
Sac2
d v
Con
c/d p/v
Gla Cae Rec

Figure 4

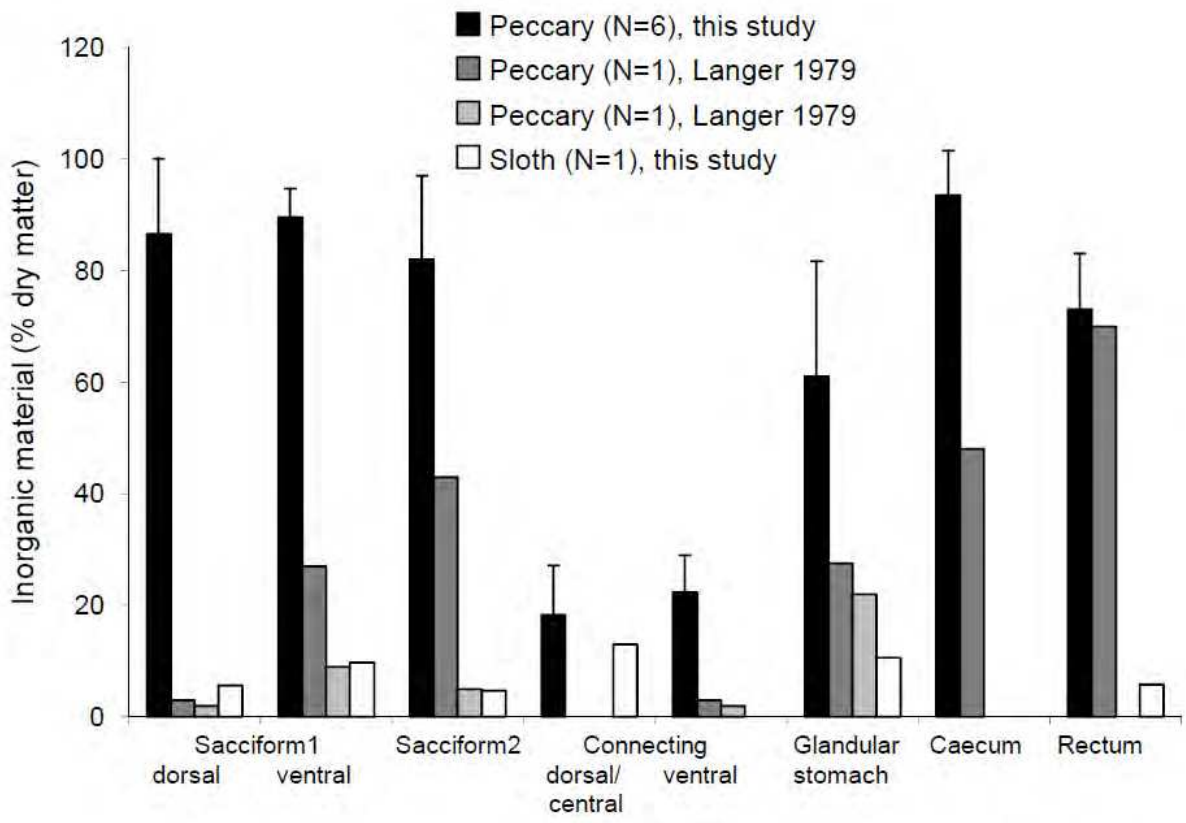

Peccary (N=6), this study
Peccary (N=1), Langer 1979
Peccary (N=1), Langer 1979
Sloth (N=1), this study
Inorganic material (% dry matter)
120
100
80
60
40
20
0
Sacciform1 dorsal
Sacciform1 ventral
Sacciform2
Connecting dorsal/ central
Connecting ventral
Glandular stomach
Caecum
Rectum

Figure 5 a

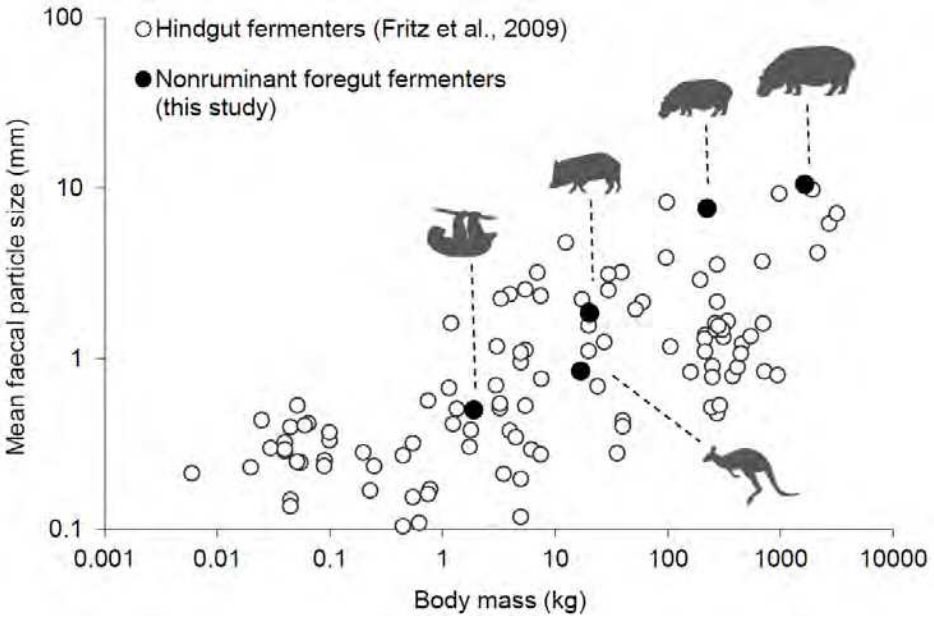


Figure 5 b